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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1759	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/574,911	AITKEN ET AL.	
	Examiner	Art Unit	
	ALEX NOGUEROLA	1759	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 October 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24 and 27-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-24 and 27-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

1. Applicant's amendment received on October 27, 2010 ("Amendment") does not render the application allowable for the reasons discussed below.

2. In regard to the rejections of claims 1-3, 6, 7, 12, 13, 17, 20, and 21 under 35 U.S.C. 102(b) anticipated by Su, Applicant states, "… Su still does not teach that a sperm type is separated from a sperm population *through* an ion-permeable barrier (emphasis added), which is required by all of the rejected claims." See page 6 of the Amendment. The Examiner respectfully disagrees. It is clearly stated throughout Su that the semen is collected at the anode. For example, see the abstract ("… the semen is collected at the anode, ..."), Claim 1 ("... the semen is collected at the anode, thus separating the X spermatozoa."), and page 4 ("… whereupon semen at the anode was collected, thus separating the X spermatozoa."). The anode can then be removed from the electrophoresis device so that the sperm collected thereon may be analysed. Thus, the sperm must move through the ion-permeable barrier (semi-permeable bag in Su)

since the electrodes are outside the ion-permeable bag in which the semen sample is initially placed. See the last paragraph on page 4 and the last paragraph on page 5 to page 7. Moreover, that sperm of a certain type move through the ion-permeable barrier is implied since the sperm collected at the anode is predominantly X type sperm (89.14% or greater), while the original sample was just slightly more than half X type (53.05 %). See Su page 6.

3. In regard to the rejections of claims 1, 5-10, 12, 13, and 17-19 under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher, Applicant argues, "The membranes described in Speicher have pores too small, less than 0.5 μm, to allow sperm to pass through. *See* Speicher, col. 5:55-65." See page 7 of the Amendment. However, Speicher does state, "Preferably, a gel membrane partition has a large pore size *that does not obstruct the movement* of charged molecules through or within the gel membrane partition. [emphasis added]" See col. 06:9-12. Also, in one example Speicher had to lessen the amount of crosslinker used to make the gel membranes and thus increase the pore size (also implied by "lower gel densities") because a large fraction of the proteins were precipitating on the separation membranes. See col. 17:50 – col. 18:02. In other words, Speicher discloses adjusting the pore size of the membranes for the desired size range of molecules or particles that are to be allowed to pass through. Since sperm typically has a diameter of 2-5 μm (see, for example, the first paragraph of page 3 of the Tilley thesis, which, although published after Applicant's

priority date, is only cited to show a property of sperm) to make the pore size of the membrane 5 μm instead of 0.5 μm is just a change in pore size within the skill of one of ordinary skill in the art. Applicant also argues in regard to Speicher, '… further review of Speicher shows that the only "cell samples" used were cell free extracts of cells or tissues …' See page 7 of the Amendment. While the Examiner acknowledges that cell free extracts of cells or tissues were used in the examples, Speicher does mention that in addition to a sample of "… a cell or tissue extract or a fraction thereof,…" the sample may be "… a biological fluid, such as serum, plasma, urine, sputum, colonic effluent, bone marrow lymph and cerebrospinal fluid" See col. 07:56-62. Additionally, making the partition membrane from polyacrylamide is disclosed in detail in Speicher (see, for example, col. 05:60-62, col. 06:04-67, and col. 17:51 – col. 18:02), which is the same membrane material Applicant used (see specification page 11, line 14: "Restriction Membrane: 10 kDa polyacrylamide"). Thus, other than the membrane pore size, which as discussed above is just an obvious change in size, there is no suggestion in the prior art that the membranes disclosed in Speicher, especially the polyacrlyamide membranes, would not be compatible with the sample in Moore.

4. In regard to the rejection of claim 11 under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher and Barbour, Applicant appears to just rely on his arguments against underlying claim 1, from which it ultimately depends. The Examiner, in turn, relies on his rebuttal above.

5. In regard to the rejection of claims 2, 3, and 14 under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher and Moore II, Applicant appears to just rely on his arguments against underlying claim 1, from which they ultimately depend. The Examiner, in turn, relies on his rebuttal above.

6. In regard to the rejection of claims 14 and 15 under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher, Jaspers, Raptis, and Burke, Applicant appears to just rely on his arguments against underlying claim 1, from which they ultimately depend. The Examiner, in turn, relies on his rebuttal above.

7. In regard to the rejections of claims 1-10, 12-23, 28, and 29 under 35 U.S.C. 103(a) as being unpatentable over Engelmann in view of Weber, Applicant states, '… Applicants pointed out that Engelmann discloses or suggests that the motility of sperm separated by free flow electrophoresis is "greatly reduced." See Engelmann, page 156. The Applicants further argued that one skilled in the art would expect even more damage to separated sperm if an ion-permeable membrane was used to separate the sperm' See page 11 of the Amendment. However, as noted in the rejection of

claim 28 in the previous Office action, 'it should be kept in mind that Applicant did not measure a change in motility other than whether the sperm moved or did not move: "Slides were scored immediately after preparation, with *any directional movement* of cells being *classified as motile* in contrast to totally immotile sperm. [emphasis added]" See Applicant's specification page 12, lines 5-11.' Thus, Applicant's claim 28 could be interpreted that at least 84 % (94% - 10%) of the separated sperm are still motile *to any extent* after the separation. While, Englemman found that the sperm had greatly reduced motility after the separation they were *still motile*. Moreover, even if Applicant's assessment of Engelmann in regard to motility is accurate these are moot points. In addition to claim 28 only Applicant's claim 4 refers to motility and it is one of several sperm characteristics in the alternative. Applicant's claims 22 and 23 state "… wherein at least about 70% [80% for claim 23] of the sperm type remains viable *or* substantially unchanged after separation." Engelmann states, "On the other hand, the *viability*, determined by eosin staining, *exhibited only an insignificant decrease* after the separation procedure. The *viability* in the different fractions *remained approximately the same* when compared with their original values before separation (Fig. 3). [emphasis added]" See Engelmann page 156. Thus, Engelmann meets claims 22 and 23, at least in regard to the alternative of whether the sperm type remains viable. Applicant's claim 29 states, "… wherein at least about 50% of the sperm type remains substantially unchanged after separation." As noted, though, (1) Englemann found that the sperm viability was substantially unchanged after separation, and (2) it appears that Applicant

did not determine relative change in motility after separation, as did Engelmann, but only whether the sperm were still mostly motile to any degree after separation.

8. In regard to the rejection of claim 11 under 35 U.S.C. 103(a) as being unpatentable over Engelmann in view of Weber and Barbour, Applicant appears to just rely on his arguments against underlying claim 1, from which it ultimately depends. The Examiner, in turn, relies on his rebuttal above.

9. In regard to the rejection of claims 18 and 19 under 35 U.S.C. 103(a) as being unpatentable over Su in view of Christensen, Applicant appears to just rely on his arguments against underlying claim 1, from which they ultimately depends. The Examiner, in turn, relies on his rebuttal above.

10. In regard to the rejection of claim 27 under 35 U.S.C. 103(a) as being unpatentable over Kricka, Applicant appears to just rely on his arguments against underlying claim 1, from which it ultimately depends. The Examiner, in turn, relies on his rebuttal above.

11. In regard to the rejection of claim 27 under 35 U.S.C. 103(a) as being unpatentable over Engelmann as modified by Weber and Kricka, Applicant appears to just rely on his arguments against underlying claim 1, from which it ultimately depends. The Examiner, in turn, relies on his rebuttal above.

12. In regard to the rejection of claim 2 under 35 U.S.C. 112, first paragraph, as not being enabled for the full scope of the claim Applicant asserts, 'The Examiner is improperly reading limitations into the claim that do not exist and then alleging that these non-existent limitations are not enabled. Claim 2 does not recite or require that a sperm is separated "based on" genetic makeup or morphological normality.' See page 13 of the Amendment. The Examiner respectfully disagrees. Claim 1 is directed to "A process for separating a sperm type from a sperm population …" Claim 2, which depends from claim 1, requires that the sperm type of claim 1 "… has a desired

characteristic selected from … genetic makeup, morphological normality, …" Thus, a fair, plain reading of claim 2 is -- A process for separating sperm type having a desired genetic makeup or morphological normality from a sperm population ... -- That is, claim 2 *does* require that a sperm be separated based on genetic makeup or morphological normality. On the other hand, even if the Examiner were to agree that claim 2 does not require a sperm to be separated based on genetic makeup or morphological normality, this would raise the question as to whether claim 2 further limits claim 1 at all, as Applicant seems to argue that claim 2 only refers to a optional or chance occurrence, that is, that if the process of claim 1 is performed, it may just happen that a sperm will be separated based on genetic makeup or morphological normality.

Applicant also refers to specification paragraph 111 for indicating "… that a statistically significant higher proportion of the spermatozoa with normal morphology were found in the separate fraction as compared to the excluded fraction ($p<0.001$)." See page 14 of the Amendment. The Examiner is not persuaded. A viewing of Figure 1 shows a difference of "% normal sperm" in the separated fraction from the excluded fraction before the electrophoresis even began (time 0 sec). This raises a question as what "separated" means since electrophoresis separation has not yet occurred. Also, has whatever separation, exclusion, or other sample preprocessing that has been performed on the samples relevant to Figure 1 before electrophoresis brought about the initial difference in "% normal sperm"? Additionally, another viewing of Figure 1 shows that at least three time periods into the electrophoresis, at 120 sec, 300 sec, and 900 sec, the % normal sperm in the "normal separated sample" actually *dropped* from what

it was initially at time 0 sec. Applicant is invited to provide the % of the separated fraction with normal morphology at t = 0 sec and the % of the excluded fraction with normal morphology at t = 0 sec so that a better determination can be made of the effectiveness of 5 minutes using the claimed electrophoresis process.

13. In regard to the rejection of claim 4 under 35 U.S.C. 112, first paragraph, as not being enabled for the full scope of the claim 'Applicant again respectfully assert, that the "based on" limitation is being improperly read into the claim.' See page 15 of the Amendment. The Examiner respectfully disagrees. Claim 1 is directed to "A process for separating a sperm type from a sperm population …" Claim 4, which depends from claim 1, requires that the sperm type of claim 1 "… has an undesired characteristic selected from … high levels of DNA damage and high levels of reactive oxygen species generation, …" Thus, a fair, plain reading of claim 4 is -- A process for separating sperm type having an undesired high level of DNA damage or high level of reactive oxygen species generation from a sperm population ... -- That is, claim 4 *does* require that a sperm be separated based on an undesired high level of DNA damage or high level of reactive oxygen species generation. On the other hand, even if the Examiner were to agree that claim 4 does not require a sperm to be separated based on an undesired high level of DNA damage or high level of reactive oxygen species

generation, this would raise the question as to whether claim 4 further limits claim 1 at all, as Applicant seems to argue that claim 4 only refers to a optional or chance occurrence, that is, that if the process of claim 1 is performed, it may just happen that a sperm will be separated based on an undesired high level of DNA damage or high level of reactive oxygen species generation.

Applicant also refers to specification paragraph 113 for indicating "… and in paragraph 113, the excluded fraction had significantly higher levels of DNA damage ($p<0.001$)." See page 15 of the Amendment. The Examiner is not persuaded. For one thing the error margins are so large they led to peculiar possible results for the method. If the separated population is 6.5 % (4.5 + 2.0 %) sperm with detectable DNA damage and the excluded sperm population is 11.9% (8.3 + 3.6%) sperm with detectable DNA damage than the separated population will have a lower percentage of sperm with detectable DNA damage. However, if the separated sperm population is the same (6.5% with detectable damaged), but the excluded population is 4.7 % (8.3-3.6%) sperm with detectable DNA damage than the separated sperm population will actually have a *higher* percentage of sperm with detectable DNA damage than the excluded sperm population. Also, a viewing of Figure 3 shows a difference of "% detectable DNA damage" in the separated fraction from the excluded fraction before the electrophoresis even began (time 0 sec). This raises a question as what "separated" means since electrophoresis separation has not yet occurred. Additionally, has whatever separation, exclusion, or other sample preprocessing that has been performed on the samples relevant to Figure 3 before electrophoresis brought about the initial difference in "%

detectable DNA damage"? Applicant is invited to provide the % of the separated fraction with detectable DNA damage at t = 0 sec and the % of the excluded fraction with detectable DNA damage at t = 0 sec so that a better determination can be made of the effectiveness of 5 minutes using the claimed electrophoresis process.

Regarding the alternative in claim 4 of separating a sperm type having high levels of reactive oxygen species generation, Applicant refers to the article attached as Exhibit A to the Amendment for showing that it was known at the time of the invention how to use a chemiluminescence assay for detecting reactive oxygen species generation from sperm. However, while Applicant does not have to describe what was known at the time of the invention for enablement, Applicant does have to show that at the time of the invention he contemplated using the method described in Exhibit A or at least that it was a standard or very common procedure at the time of the invention. Other than claim 4 and specification paragraph [0012], only in passing, there is no other mention in the original disclosure about levels of reactive oxygen in sperm. Even if Applicant can show that Figure 4 concerns electrophoretically separating out a sperm type having high levels of reactive oxygen species generation, Applicant has not addressed the points raised at the bottom of page 24 of the previous Office action. In any event Exhibit A does not shed any light on how to use the *electrophoresis method* for separating sperm with a high level of reactive oxygen species from sperm without a high level of reactive species generation. For this Applicant relies on the Exhibit B article, particularly Figure 3 and its related discussion. As a first matter, as acknowledged by Applicant, Exhibit B has a post-effective-filing date. Second, the

Examiner has not found any mention in Exhibit B about determining levels of reactive oxygen species generation in sperm. Figure 3 concerns "[z]ymosan elicited chemciluminescence responses generated by populations of spermatozoa in the presence of luminol and horse-radish peroxidase." See the Figure 3 caption. This is apparently only related to the number and quality of spermatozoa. See Results – *Number and quality of spermatozoa*, which begins on page 2264. Last, there is no mention of "zymosan" in the Exhibit A article. Thus, putting aside new matter concerns, there is a question now of which method would be used to detect the level of reactive oxygen species in sperm, the one disclosed in Exhibit A or the one supposedly disclosed in Exhibit B.

Status of Rejections pending since the Office action mailed on June 11, 2010

14. All of the prior art rejections, that is, rejections based on 35 U.S.C. 102(a) and 35 U.S.C. 103(a), are withdrawn, but have been largely restated, below, as previously presented, the only changes being in the various rejections of claim 1, in light of Applicant's Amendment.

15. The scope of enablement rejections of claims 2 and 4 are

Claim Rejections - 35 USC § 102

16. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

17. Claims 1-3, 6, 7, 12, 13, 17, 20, and 21 are rejected under 35 U.S.C. 102(b) as being anticipated by an English language translation of CN 89105110.4 (hereafter "Su").

Addressing claim 1, Su discloses a process for separating a sperm type from a sperm population in a sperm sample by electrophoresis (claim 1) comprising subjecting the sperm population to an electric potential such that a sperm type is separated from a sperm population through an ion-permeable barrier. See the last paragraph on page 4 of Su. The Examiner construes the "semi-permeable bag" of Su as the claimed "ion-permeable barrier" because Su states, "Due to the separation method of this invention, the design of the electrophoresis cell used can prevent direct contact between the semen and the electrodes while also supporting the spermatozoa's source of energy without affecting the spermatozoa." See the tip of page 5. If the semi-permeable bag were not permeable to ions then electrolyte would be blocked and electrophoresis field disrupted.

While Su does not specifically state "… that a sperm type moves through the ion-permeable barrier and is separated from a sperm population through the ion-permeable

barrier", this inherently occurs withy the method of Su. It is clearly stated throughout Su that the semen is collected at the anode. For example, see the abstract ("… the semen is collected at the anode, ..."), Claim 1 ("... the semen is collected at the anode, thus separating the X spermatozoa."), and page 4 ("… whereupon semen at the anode was collected, thus separating the X spermatozoa."). The anode sperm can then be removed from the electrophoresis device so that the collected sperm may be analysis. As such the sperm must have moved through the ion-permeable barrier (semi-permeable bag in Su) since the electrodes are outside the ion-permeable bag in which the semen sample is initially placed. See the last paragraph on page 4 and the last paragraph on page 5 to page 7. Moreover, that sperm of a certain type move through the ion-permeable barrier and is separated from a sperm population through the ion-permeable barrier is implied since the sperm collected at the anode is predominantly X type sperm (89.14% or greater), while the original sample was just slightly more than half X type (53.05 %). See Su page 6.

Addressing claims 2 and 3, Su discloses at least separating sperm type having a certain gender and robustness or fertilizing potential (e.g., "In the results of ten experiments on X spermatozoa from semen by the method of this invention, the average value was 91.79 ± 6.4, with vitality of 0.68 ± 0.18." See bottom of page 6.

Addressing claim 6, the semen sample explicitly contains X-sperm and implicitly Y-sperm.

Addressing claims 7, 20, and 21, for the additional limitations of these claims see the bottom of page 6 ("In the results of ten experiments on X spermatozoa from semen by the method of this invention, the average value was 91.79 ± 6.4, with vitality of 0.68 ± 0.18.").

Addressing claims 12 and 13, for the additional limitations of these claims see the last paragraph on page 4 ("… at a voltage 3V and a current of 40-100 μA, …")

Addressing claim 17, for the additional limitation of this claim see page 6 ("The semen was diluted with 3.9 mL of diluant.")

Claim Rejections - 35 USC § 103

18. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

19. Claims 1, 5-10, 12, 13, and 17-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moore et al. "Isoelectric Focusing of Boar Spermatozoa," *J. Reprod. Fert.* (1975) 44, 329-332 (hereafter "Moore"), Speicher et al. US 6,638,408 B1 (hereafter "Speicher").

Addressing claim 1, Moore discloses a process for separating a sperm type from a sperm population in a sperm sample by electrophoresis (abstract and Figure 1) comprising subjecting the sperm population to an electric potential such that a sperm type is separated from a sperm population through a pH-gradient (abstract; Figure 1; and last paragraph on page 329, bridging to page 330). Moore does not mention providing an ion-permeable membrane in the isoelectric focusing device.

Speicher discloses a method and device for separation of charged molecules by solution isoelectric focusing. The device comprises a separation chamber having an electrode at each end thereof between which is a series of ion-preamble barriers spaced apart in sequence that partition the chamber into regions having a preset pH range. See the abstract; Figures 1, 8, and 9; col. 04:56 - col. 05:17; and col. 06:17-31. It would have been obvious to one with ordinary skill in the art at the time of the invention to substitute the isoelectric focusing device of Speicher for that used by Moore in Moore's method because the isoelectric device of Speicher does not require a large sample volume; does not produce large volume, dilute fractions that need to be concentrated with attendant loses; has very good resolution; and does not require

expensive, complex instrumentation. See Speicher col. 02:30-37. Additionally, the device of Speicher would be easier to prepare than that of Moore because a pH density solution (ficoll density gradient) does not have to be prepared as instead solid pH membranes are used. As such, the isoelecric focusing device of Speicher potentially offers much better separation than the device of Moore because the pH intervals in Speicher can be much more easily controlled just by adjusting the number of, thicknesses of, and spacing between the pH membrane, rather than trying to distribute the Ampholines in the ficoll density into very narrow bands. See col. 06:04-61 and col. 07:12-17. Furthermore, sample recovery is also much easier with the isoelectric focusing device of Speciher than with that of Moore. Compare col. 07:18-23 in Speicher with "Elution of the gradient was by way of a 'flow-through' pH electrode" and "The gradient containing the focused spermatozoa was run from the column through a 0.5 ml 'flow-through' pH electrode connected to a pH meter …" as discussed in the Figure 1 caption and top paragraph on page 330 of Moore. Although Speicher has a preferred ion-permeable barrier pore size of less than 0.5 microns Speicher does teach adjusting the pore size so that the desired particles can pass through. See col. 06:04-16 and col. 17:51 – col. 18:02. Finally, Applicant should note that although Speicher mostly describes using the isoelectric focusing device with samples of protein mixtures, Speicher does discloses that the samples may be biological fluids or cell or tissue samples. See col. 10:33-37 and col. 07:56-62. Thus, to use the isoelectric focusing device of Speicher to practice the method of Moore (that is substituting the Perspex column and ficoll pH gradient of Moore with the isoelectric focusing device of Speicher

having equivalent or even narrower pH intervals and still using the same electrode solutions and sample) is just substitution of one known device for another with predictable results.

Addressing claim 5, for the various claimed sample chambers, electrolyte chambers, and ion-permeable barriers see Figures 1, 8, and 9 in Speicher.

Addressing claim 6, Moore used at least spermatozoa from intact boars and spermatozoa from vesiculectomized boars. See the last paragraph on page 330.

Addressing claim 7, for the additional limitation of this claim see Figure 2 in Moore, for example.

Addressing claims 8-10, for the additional limitations of these claims see in Speicher col. 05:36-54, which discloses that a range of range of small diameter pore sizes could be used. Barring a contrary showing, such as unexpected results, the choice of pore size is just optimization of the separation. As for the ion-permeable barriers being "electrophoresis membranes", since they are being used in an electrophoresis

device they are "electrophoresis membranes". Moreover, Speicher discloses making the membranes from a variety of materials including polyacrylamide, agarose, or polyacrylamide-agarose. See col. 05:55-61.

Addressing claim 12, although Moore does not mention any voltage range Speicher discloses using 100 V and 200 V to separate cellular material. See col. 16:29-44. Thus, barring a contrary showing, such as unexpected results to carry out the electrophoresis within the claimed voltage is range is optimization of known result effective separation variable.

Addressing claim 13, for the additional limitation of this claim see page 330 of Moore, which discloses applying a 2 mA current during electrophoresis.

Addressing claim 17, for the additional limitation of this claim note that Moore discloses diluting the sperm in the pH gradient (“Spermatozoa from intact and vesiculectomized boars …resuspended in 0.3 ml of the pH/ficoll gradient removed from the Colum …” – bottom of page 329, bridging to page 330) thus Moore implicitly discloses diluting the sperm in a buffer, although molar concentration of the buffer is not clear. However, barring a contrary showing, such as unexpected results, selecting the buffer concentration is just a matter of optimization especially in an isoelectric focusing device.

Addressing claims 18 and 19, the sperm sample concentration in Moore is 5 x 10^6 / 0.3 ml, which is about 18.6 x $10^{6/}$ ml, which is within both the claimed ranges.

20. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Moore as modified by Speicher as applied to claims 1, 5-10, 12, 13, and 17-19 above, and further in view of Barbour et al. US 5,436,00 (hereafter "Barbour").

Although Speicher does not appear to mention polycarbonate as a membrane material Speicher does discloses that a variety of materials could be used including synthetic and natural polymers and glass. Speicher also discloses that the pore size may be as small as 0.5 microns. See col. 05:55-65. As shown by Barbour polycarbonate membranes with a pore size of 3 μm were used in biochemical experiments on cells at the time of the invention. See the abstract and col. 15:21-25. Thus, barring a contrary showing, such as unexpected results, to have the membrane be made of polycarbonate and have a pore size within the claimed range is just use of known membrane material and pore size in related biochemical/biological arts for optimizing the sperm separation during electrophoresis.

21. Claims 2, 3, and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher as applied to claims 1, 5-10, 12, 13, and 17-19 above, and further in view of Moore "The Net Surface Charge of Mammalian Spermatozoa as Determined by Isoelectric Focusing, Changes Following Sperm Maturation, Ejaculation, Incubation in the Female Tract, and after Enzyme Treatment" International Journal of Andrology 2 (1979) 449-452 (hereafter "Moore II").

Addressing claims 2 and 3, arguably Moore already discloses at least separating sperm type having fertilizing potential since normal boar spermatozoa could be differentiated by isoelectrophoresis from spermatozoa from boar without seminal vesicles. See the Moore abstract. In any event, Moore II teaches that with isoelectric focusing "… a preliminary study showed that spermatozoa from some apparently infertile men have an isoelectric point consistently higher than those from fertile men." See page 449. Thus, in light of Moore II to use the process of Moore as modified by Speicher to separate sperm based on fertilizing potential is an obvious variant that would be clearly useful in determining and diagnosing infertility.

22. Claims 14 and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Moore in view of Speicher as applied to claims 1, 5-10, 12, 13, and 17-19 above, and further in view of Jaspers et al. "Separation of Bacterial Cells by Isoelectric Focusing, a New Method for Analysis of Complex Microbial Communities," Applied and Environmental Microbiology, Aug. 1997., p. 3176-3181 (hereafter "Jaspers") and Raptis US 6,001,617 (hereafter "Raptis"), Burke, Jr. et al. US 2002/0119218 A1 (hereafter "Burke")

Addressing claims 14 and 15, Moore does not appear to mention the voltage gradient applied during electrophoresis. However, barring a contrary showing, such as unexpected results, adjusting the voltage gradient is just optimization of a known result effective variable. As was known in the art at the time of the invention the higher the electrophoresis voltage the faster the separation; however, this gain is offset by increased heating of the electrophoresis solution, which may distort the separation. In fact, too high a voltage gradient, which may porate, fuse, injure, or even kill living cells. See, for example, Raptis col. 02:60-65; Burke the abstract and specification paragraph [0011]. It was also known at the time of the invention to use a voltage gradient of 11.5 V/cm for isoelectrophoresis of living whole bacterial cells, which would further suggest to one of ordinary skill in the art at the time of the invention to use a low voltage gradient, such as within the claimed ranges. See the Jaspers abstract and the second column on page 3179.

23. Claims 1-10, 12-23, 28, and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Engelmann et al. "Separation of Human X and Y Spermatozoa by Free-Flow Electrophoresis," Gamete Research 19:151-159 (1988) (already of record, hereafter "Engelmann") in view Weber US 7,3999,394 B2 (hereafter "Weber")

Addressing claim 1, Engelmann discloses a process for separating a sperm type from a sperm population in a sperm sample by free-flow electrophoresis (abstract) comprising subjecting the sperm population to an electric potential such that a sperm type is separated from a sperm population through a buffer medium (abstract). Engelmann does not mention providing an ion-permeable membrane in the isoelectric focusing device.

Weber discloses a free-flow electrophoresis method and device for separating charged substances in which sample components pass through one or more ion-permeable barriers (2) arranged parallel to the electrodes (4). See the abstract; Figures 3-6; and col. 03:38-47. It would have been obvious to one with ordinary skill in the art at the time of the invention to provide ion-permeable barriers as taught by Weber in the invention of Engelmann because as taught by Weber this will enhance sample separation by combining electrofiltration with free-flow fractionation in a way that avoids overheating of the fluid in the device, yet allows increased sample throughput at high speed. See col. 01:17 - col. 02:40; and col. 05:15-45.

Addressing claims 2-4, Engelmann discloses at least separating sperm type based on motility, robustness, fertilizing potential, or gender (the Examiner construes "viability" in Engelmann to encompass robustness and fertilizing potential). See the abstract, Y-Chromatin Distribution Before and After Separation on page 154, and Viability and Motility of Separated Spermatozoa on page 156.

Addressing claim 5, for the various claimed sample chambers, electrolyte chambers, and ion-permeable barriers see Figure 6 in Speicher.

Addressing claim 6, Engelmann used at least X-sperm/ Y-sperm, viable sperm/non-viable sperm, and motile sperm/ low motility sperm. See pages 153 and 156.

Addressing claim 7, for the additional limitation of this claim see in Engelmann Figure 2 and Table 1, for example.

Addressing claims 8-10, for the additional limitations of these claims consider that Weber discloses using the membranes for electrofiltration, which clearly suggests that the pore size would be pre-selected. This also clearly stated in col. 03:55-57: "The material and the pore size of the hollow fiber 2 differ according to the application

concerned, i.e., the samples to be treated, and are chosen accordingly." With multiple membranes as shown in Figure 6 they clearly do not all have to have the same pore size. For claim 9 note that since the membranes in the device used in the process of Engelmann as modified by Weber is an electrophoresis device, the membranes are "electrophoresis membranes" in addition to being ion-permeable membranes.

Addressing claims 12 and 14 for the additional limitation of this claim see in Engelmann Electrophoresis: Operating Conditions on page 153.

Addressing claim 13, Engelmann as modified by Weber does not appear to mention a current or current range during electrophoresis; however, barring a contrary showing, such as unexpected results, adjusting the current is just a matter of adjusting a known result-effective variable for optimization of the separation. If the current is too high, which would primarily be due to the voltage gradient and the conductivity of the electrophoresis solution, then there may be overheating, which would adversely affect the separation. Also, too high a current may actually kill the sperm cells. On the other hand, too low a current suggests too low a voltage and too slow a separation.

Addressing claim 15, Engelmann only mentions a voltage gradient of 80-120 V/cm. See Engelmann Electrophoresis: Operating Conditions on page 153. However,

Engelmann states, "The velocity of the chamber buffer flow was adapted to each individual sample and the selected electric current." *ibid.* The voltage gradient will largely decide the current along with conductivity of the electrophoresis solution. One with ordinary skill in the art at the time of the invention would recognize that the voltage gradient could be reduced, but at the cost of increasing the separation or adversely affecting the quality of the separation. Thus, barring a contrary showing, especially since no other operational parameters are claimed, to reduce the voltage gradient from 80 V/cm as already taught by Engelmann to 16 to 20 V/cm as claimed will have predictable results.

Addressing claim 16, Engelmann discloses an electrophoresis time of 80 seconds. As for the sample size it must be very small, such as that claimed, because the separation chamber is only 120 x 30 x 0.3 mm. See Electrophoresis: Operating Conditions on page 153 and Free-Flow Electrophoresis on page 152.

Addressing claim 17, Engelmann states, "Briefly, the ejaculate was diluted twofold with Hanks' solution (Boehringer Mannheim, buffered with HEPES 10 mM, …"). See Buffer Systems on page 152.

Addressing claims 18 and 19, Engelmann states, "… to give a final sperm density of approximately 60-80 x 10^6 /ml." See Free-Flow Electrophoresis on page 152.

Addressing claims 20-23 and 29, Engelmann states, "On the other hand, the viability determined by eosin staining, exhibited only an insignificant decrease after the separation procedure. The viability in the different fractions remained approximately the same when compared with their original values before separation (Fig. 3)." See Viability and Motility of Separated Spermatozoa on page 156.

Addressing claim 28, although Engelmann found reduced motility for each sperm fraction (Viability and Motility of Separated Spermatozoa on page 156), the fractions were still motile (Figure 3 for example has a lowest motility between 5-10%). In regard to this claim, it should be kept in mind that Applicant did not measure a change in motility other than whether the sperm moved or did not move: "Slides were scored immediately after preparation, with *any directional movement* of cells being *classified as motile* in contrast to totally immotile sperm. [emphasis added]" See Applicant's specification page 12, lines 5-11. In contrast, Engelmman still found sperm movement, albeit reduced.

Claim Rejections - 35 USC § 112

24. Claim 2 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for separating sperm based on motility, robustness, gender, and fertilizing potential does not reasonably provide enablement for separating sperm based on genetic makeup or morphological normality. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with this claim.

Genetic makeup is determined by chromosomes contained within the sperm cell. More particularly it is the DNA sequence of the chromosomes that determines genetic makeup ("Each gene has its own specific location on the chromosome and is a piece of genetic material that does one particular job. All of the 20,000 or so genes contain a different 'packet' of information necessary for out bodies to grow and work. Our genes also contain the information for how we look: the colour of our eyes, how tall we are, the shape of our nose, etc. The information is in the form of a chemical (DNA) code (the genetic code) .."). See the "Genes and Chromosomes" article produced by the Centre for Genetics Education, especially page 4. The Examiner is not aware of any correlation between sperm movement under electrophoresis and the DNA sequence of chromosomes therein. Indeed, it would be most astounding if the DNA sequence of

chromosomes could be sequenced intact in sperm simply by moving them with an electrical field through one or more membranes. Moreover, there does not appear to be any mention in the original specification of results of a genetic analysis of intact sperm by electrophoresis, let alone an example or suggestion as how to carry out such an analysis.

With regard to separating sperm based on morphological normality, Applicant's own specification discloses that Applicant has not been successful in such a separation. Figure 1 shows that at every electrophoretic time point comparable amounts of both groups of sperm appeared. Indeed, Applicant found "… normal morphology in the separated fraction (35±1.2%) compared to that of the excluded fraction (28±4%) …" 35% - 1.2% = 33.8%. 28% + 4% = 32 %. 33.8% - 32% = *1.8%,* which is not a significant difference. See page 15 of the specification. Moreover, the sperm underwent considerable chemical and biochemical processing after being collected at the different electrophoresis time points. See Sperm Morphology, which begins on page 13 of the specification. It would seem, therefore, that the electrophoresis had little if any role in distinguishing sperm based on morphological normality.

For these reasons undue experimentation would be required to practice the invention of claim 2 for genetic make-up and morphological normality, so Applicant's disclosure does not enable "genetic makeup" and "morphological normality" in claim 2.

25. Claim 4 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for separating sperm based on poor motility, does not reasonably provide enablement for separating sperm based on poor morphology, high levels of DNA damage, and high levels of reactive oxygen species generation. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with this claim.

The issue of enablement with respect to separating sperm by motility has already been addressed above in the rejection of claim 2 under 35 U.S.C. 112, first paragraph. So those arguments will also apply here without being repeated.

With regard to separating sperm based on high levels of DNA damage, Figure 3 purports to show some sort of separation of sperm with damaged DNA from sperm with undamaged DNA. See page 5. However, the greatest separation in the two groups occurred at time zero, presumably before electrophoresis even began. Additionally, the absolute and relative amounts did not vary much over time when one considers that the tallest bar is not over 10%. As for the claim in claim 2 of being able to separate sperm based on genetic make-up, it would be remarkable DNA damage of DNA in intact living sperm cold be detected just by using an electric field to move them one or more membranes.

With regard to separating sperm based on high levels of reactive oxygen species generation. Results shown in Applicant 's Figure 4 do not show clear separation of sperm with high level of reactive species oxygen species generation from sperm without

high levels of reactive oxygen species generation *using electrophoresis*. Indeed, the greatest separation appears at time 0 (zero), before electrophoresis. Also, the samples collected at the different time points underwent considerable mechanical, chemical, and biochemical processing *after* the electrophoresis. See Acrosmoe Reactions on page 14 of the specification. So, electrophoresis seems to have played little role in the sperm separation based on levels of reactive oxygen species generation.

26. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

27. Claims 2 and 4 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

a) Claim 2 – Although the claim language strongly suggests otherwise, Applicant argues that claim 2 does not require the separation of a sperm based on genetic makeup or morphological normality. Applicant instead appears to argue that whether a separated sperm has one of the listed characteristics, such as one of the two just noted, is a chance occurrence. As such, claim 2 does nor appear to further limit the process of claim 1. Applicant is requested to more clearly explain (and if possible amend) claim 2 so one may understand how claim 2 is actually intended to further limit claim 1.

b) Claim 4 – Although the claim language strongly suggests otherwise, Applicant argues that claim 4 does not require the separation of a sperm based on an undesired high level of DNA damage or high level of reactive oxygen species generation. Applicant instead appears to argue that whether a separated sperm has one of the listed characteristics, such as one of the two just noted is a chance occurrence. As such, claim 4 does nor appear to further limit the process of claim 1. Applicant is requested to more clearly explain (and if possible amend) claim 4 so one may understand how claim 4 is actually intended to further limit claim 1.

Final Rejection

28. Applicant's amendment necessitated the new grounds of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

29. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ALEX NOGUEROLA whose telephone number is (571) 272-1343. The examiner can normally be reached on M-F 8:30 - 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ALEXA NECKEL can be reached on (571) 272-1446. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Alex Noguerola/
Primary Examiner, Art Unit 1759
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